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NP-40 Lysis Buffer

1 Contents

Component	HY-K1002-100 mL	HY-K1002-500 mL
NP-40 Lysis Buffer	100 mL	100 mL × 5

2 Introduction

MCE NP-40 Lysis Buffer is a relatively mild lysis solution that can be used for animal and plant cells or tissues, as well as fungal and bacterial samples. The protein samples obtained by lysis can be used for PAGE, WB, IP, Co-IP, ELISA and other experiments, etc. Mild lysis is benefit for maintaining the original protein structure and the original protein-protein interactions.

MCE NP-40 Lysis Buffer contains 50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40 and a variety of inhibitors such as sodium pyrophosphate, β-glycerophosphate, sodium orthovanadate, sodium fluoride, EDTA and leupeptin, etc., which can effectively inhibit protein degradation.

3 Protocol

Pipette proper volume of NP-40 Lysis Buffer and mix it gently, put it on ice for using.

Note: Add Protease Inhibitor Cocktail to lysis buffer to prevent proteolysis. Add Phosphatase Inhibitor Cocktails to maintain phosphorylation status of proteins as needed (Recommended product list). Incubate on ice for subsequent use.

1. For cell sample

For adherent cells

1) Carefully remove culture medium from adherent cells.

2) Discard residual medium, wash cells one or two times with cold Wash Buffer, such as PBS, normal saline or Serum-Free Culture Medium.

3) Add proper volume of cold NP-40 Lysis Buffer (eg. 150-250 μL per well for 6-well plate), then stroke with pipette until the buffer immerses cells completely.

Note: Incubate on ice and shake slightly for 1-2 seconds for animal cells, or 2-10 minutes for plant cells.

4) After lysis, centrifuge at 10,000-14,000 g for 3-5 minutes, transfer the supernatant to a new tube for subsequent analysis, such as PAGE, WB and IP, Co-IP, etc.

For suspension cultured cells

1) Collect cells. Centrifuge at 500 g for 5 minutes and discard the supernatants.

Note: Washing cells one or two times with cold Wash Buffer before collecting cells is recommended, such as PBS, normal saline or Serum-Free Culture Medium.

2) Add proper volume of cold NP-40 Lysis Buffer (eg. 150-250 μL per well for 6-well plate).

Note: Flick the tube bottom to aid the cell disruption.

3) After lysis, centrifuge at 10,000-14,000 g for 3-5 minutes, transfer the supernatant to a new tube for subsequent analysis, such as PAGE, WB and IP, Co-IP, etc.

For Bacteria or yeast cells

For better lysis results, bacteria and yeast can be digested with lysozyme and lyticase, respectively, before using NP-40 Lysis Buffer.

1) For 1 mL of bacterial or yeast cells, centrifuge and discard the supernatant.

Note: Washing cells one or two times with cold Wash Buffer before collecting cells is recommended, such as PBS, normal saline or Serum-Free Culture Medium.

2) Add 100-200 μL of NP-40 Lysis Buffer, incubate on ice and shake slightly for 2-10 minutes.

3) After lysis, centrifuge at 10,000-14,000 g for 3-5 minutes, transfer the supernatant to a new tube for subsequent analysis, such as PAGE, WB and IP, Co-IP, etc.

2. For tissue sample

1) Cut tissue sample into small pieces on ice quickly to minimize protein degradation.

2) Add 150-250 μL cold NP-40 Lysis Buffer per 20 mg of tissue sample and homogenize using electric homogenizer. Add more Lysis buffer if tissue is not completely lysed.

3) After lysis, centrifuge at 10,000-14,000 g for 3-5 minutes, transfer the supernatant to a new tube for subsequent analysis, such as PAGE, WB and IP, Co-IP, etc.

Note: 20 mg of frozen mouse liver tissues may yield 15-25 mg/mL protein (for reference).

4 Storage

Store at -20°C for 1 year.

5 Precautions

1. This product should avoid repeated freezing and thawing, aliquot the stock solution to routine usage volumes and store at -20°C.
2. All steps should be performed either on ice or at 4°C.
3. Do not add phosphatase inhibitors when preparing lysates for phosphatase assays.
4. Use BCA Protein Assay kit to quantify lysed proteins. Bradford Protein Assay kit is not recommended.
5. It is sufficient to add 150 μL of NP-40 Lysis Buffer to each well of 6-well plate with 0.5-5 × 10^6 cells or 1 mL of bacterial solution or yeast solution. The volume of NP-40 Lysis Buffer can be increased to 200-250 μL if the cell density is large.
6. The protein concentration is about 2-4 mg/mL for every 1 × 10^6 animal cells after lysing with 100 μL of NP-40 Lysis Buffer (for reference).
7. This product is for R&D use only, not for drug, household, or other uses.
8. For your safety and health, please wear a lab coat and disposable gloves to operate.

Recommended products used for Protein Sample Preparation

Cat. No.	Product Name	Application
HY-K1001	RIPA Lysis Buffer (Strong)	Cell Lysis
HY-K1002	NP-40 Lysis Buffer	
HY-K0010	Protease Inhibitor Cocktail	Protease Inhibitor Cocktail
HY-K0011	Protease Inhibitor Cocktail, mini-Tablet	
HY-K0012	Protease Inhibitor Cocktail, Bacteria (EDTA-Free, 100× in DMSO)	
HY-K0013	Protease and Phosphatase Inhibitor Cocktail (EDTA-Free, 10× in ddH_2O)	
HY-K0021	Phosphatase Inhibitor Cocktail I	Phosphatase Inhibitor Cocktail
HY-K0022	Phosphatase Inhibitor Cocktail II	
HY-K0023	Phosphatase Inhibitor Cocktail III	
HY-K0030	Deacetylase Inhibitor Cocktail	Deacetylase Inhibitor Cocktail
HY-K0016	Kinase Inhibitor Cocktail (5×, in ddH_2O)	Kinase Inhibitor Cocktail
HY-K3005	PBS Buffer (1×)	Wash Buffer
HY-K1023	PBS Powder (1 L of 1×)	